# Changes in Photosynthesis and Leaf Characteristics from Seedlings to Mature Canopy Individuals of Some Dipterocarp Species in a Tropical Rain Forest, Sarawak, Malaysia

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## Introduction

Tropical rain forests have a complex and multilayered vertical structure. The crown surface of emergent trees in tropical rain forests usually experiences strong sunlight, whereas less than 1% of the solar radiation typically reaches the forest floor (Chazdon 1988). How do tree leaves respond to such drastic differences in light conditions at differing heights? It is well known that leaves in bright conditions (sun leaves) have greater nitrogen content and leaf mass per unit area (LMA), corresponding to a higher photosynthetic rate at light saturation ($A_{max}$), than leaves in dark conditions (shade leaves). Shade leaves have a higher chlorophyll content and thinner leaf blade to maintain the dark respiration rate. Thus, the light compensation point ($I_c$) of shade leaves is smaller than that of sun leaves (Lambers et al. 1998). Carswell et al. (2000) and Rijkers et al. (2000) reported that the $A_{max}$, LMA, and leaf nitrogen content increased significantly with tree height in some neotropical forest trees, although neither report gave much information about large canopy trees or emergent trees.

Does the photosynthetic capacity of canopy and emergent trees increase with height in the tropical rain forest? Some researchers have reported that the age- and/or size-dependence physiological traits showed an ontogenetic decrease in $A_{max}$ with decreasing leaf nitrogen content and increasing of LMA in some canopy species (e.g., Thomas and Winner 2002). In general, this decline in leaf nitrogen content causes a reduction in $A_{max}$ in tall trees (Niinemets 2002). It is well known that $A_{max}$ may also depend on LMA (Thomas and Winner 2002), and an increase of LMA with tree size leads to an increase in resistance of $CO_2$ diffusion within the leaf and then a decrease of $A_{max}$ (Terashima et al. 2001).

Tropical canopy and emergent trees may, however, realize a high $A_{max}$ by developing a leaf mesophyll structure adjusted to the tropical canopy environment. Kenzo et al. (2004) reported that, in some canopy species with high $A_{max}$ values (nearly 20 μmol $CO_2$ $m^{-2}$ $s^{-1}$) in a Southeast Asian tropical rain forest, $A_{max}$ had a higher positive correlation with leaf mesophyll structure, such as leaf palisade layer thickness and surface area of mesophyll cells per unit leaf area ($A_{mes}/A_a$), than with leaf nitrogen content and LMA. Alhough an increase in tree height is usually a known cause of declining physiological function of the hydraulic architecture for the transport of water from roots to leaves, we propose and test the hypothesis that tree height does not limit $A_{max}$ for tropical canopy species in the tropical rain forest.

Our objective in the present study was to determine the effect of tree height on leaf photosynthesis, as

well as on morphological and biochemical properties, in five dipterocarp species. To enable us to sample leaves from seedlings on the dark forest floor to mature canopy trees at the bright canopy layer, we used a canopy crane system that provided three-dimensional access to the forest.

## Materials and Methods

### *Study site and plant material*

Our study was conducted in an experimental plot (4 ha, 200×200 m) in a lowland dipterocarp forest in Lambir Hills National Park, Sarawak, Malaysia (4°20'N, 113°50'E; 150 to 250 m a.s.l.) in September 2001. In the study plot, the mean height of the canopy layer in the stand was about 30 to 40 m, and some emergent trees reached 50 m. The annual precipitation and temperature at the study site averaged 2429 mm and 26.3°C from 2000 to 2003, respectively.

We selected five dipterocarp species and 65 individuals, ranging from seedlings to mature trees (Table 1). The species examined were *Dipterocarpus globosus* Vesq. (DG), *Dryobalanops aromatica* Gaertn. f. (DA), *Shorea acuta* Ashton (SA), *S. beccariana* Burck (SB), and *S. macroptera* Dyer (SM). The height of selected trees ranged from 0.6 to 53 m, and the sample included small seedlings, pole-sized saplings, and emergent trees. The seedlings and saplings were chosen both from gaps and their periphery and under a closed canopy.

### *Gas exchange measurements*

Leaf gas exchange rate was measured using a portable photosynthesis apparatus (LI-6400, Li-Cor, Lincoln, NE). All measurements were made in the morning between 0800 and 1100 in order to avoid the midday depression in photosynthesis (Kenzo et al. 2003). We selected three fully expanded and apparently non-senescing leaves taken from the top of the crown. The relation between the photosynthetic photon flux density and the carbon assimilation rate was determined. The light intensity, $CO_2$ concentration, and temperature in the chamber were controlled at 0 to 1800 μmol photon $m^{-2}$ $s^{-1}$, 360 ppm, and 30°C, respectively. Based on the measurement data, we estimated $A_{max}$ and $I_c$.

### *Leaf nitrogen content, chlorophyll determination, and leaf mesophyll structures*

Following the gas exchange measurements, all leaves were sampled, and divided into three parts: one for measuring dry weight and nitrogen content, one for chlorophyll analysis, and the other for observation of mesophyll structure. Leaf nitrogen and carbon contents were determined by an NC analyzer (Sumigraph NC-900, Shimadzu). Chlorophyll was extracted with DMSO and was determined by a spectrophotometer (UV-1400, Shimadzu). The leaves used for observation of the mesophyll structure were fixed in FAA, and transverse slices were prepared. The thicknesses of the leaf blade and palisade layer were determined. The surface area of mesophyll cells per unit leaf area ($A_{mes}/A_a$) was estimated (Nobel 1999).

## Results

### *Leaf photosynthetic, morphological, and biochemical properties in relation to tree height*

Both within and across species, $A_{max}$ and $I_c$ increased significantly with tree height (Fig. 1A, B). We did not

find significant interspecific differences in $A_{max}$ and $I_c$ with tree height (ANCOVA; $P > 0.05$), except for in the $A_{max}$ of SB in the canopy individuals.

The leaf blade thickness and LMA increased significantly with tree height (Table 2, Fig. 1C). However, interspecific differences were found between SA and other species. SA had the thickest leaf blade and highest LMA among the species studied at all tree heights (ANCOVA; $P < 0.05$). The thickness of the palisade layer and the $A_{mes}/A_a$ value also increased with tree height without interspecific differences (Table 2, Fig. 1D).

The relationship between nitrogen content per unit area ($N_{area}$) and tree height was similar to the other traits (Table 2); no significant interspecific difference was observed. The unit mass chlorophyll content ($Chl_{mass}$) and the chlorophyll to nitrogen (Chl/N) ratio decreased with height without interspecific differences (Table 2).

***Leaf mesophyll structure and photosynthetic properties in the canopy***

Leaf mesophyll structure significantly differed among species, suggesting a stronger effect on interspecific variation in canopy photosynthesis (Kenzo et al. 2004). In particular, SB leaves had the highest $A_{max}$ (18 μmol $m^{-2}$ $s^{-1}$) and the thickest palisade layer. Surprisingly, the palisade consisted of up to five or more layers (Fig. 2).

***Leaf Amax and Ic in relation to leaf characteristics***

Significant correlations were found between $A_{max}$ and LMA and between $A_{max}$ and $N_{area}$ (Table 3; both $P < 0.001$). The highest correlation coefficients were observed between $A_{max}$ and properties of leaf mesophyll structures, such as the thickness of the palisade cell layer and $A_{mes}/A_a$ (Table 3; both $P < 0.001$).

Negative correlations were found between $I_c$ and $Chl_{mass}$ and between $I_c$ and Chl/N (Table 3).

## Discussion

***Change of photosynthetic capacity and light compensation point with tree height***

Photosynthetic capacity ($A_{max}$) was not limited by tree height in these tropical canopy tree species. For dipterocarp species, $A_{max}$ bears a simple relation to tree height (Fig. 1A). Rijkers et al. (2000) also found a significant relation between $A_{max}$ and tree height in four neotropical species. The slope and intercept of their linear regression line were very similar to those in our study. These facts may be important to estimate the capacity of $CO_2$ fixation in tropical forests, although further studies are needed in diverse tropical forests.

In contrast, at the dark forest floor, the lower $I_c$ seems to contribute to maintaining a positive carbon assimilation rate (Fig. 1B). $I_c$ was less than 10 μmol photon $m^{-2}$ $s^{-1}$ for most of the non-gap seedlings in this study. Many authors have reported that photosynthetic photon flux density below the closed canopy of tropical rain forests lies in the range of 5 to 20 μmol photon $m^{-2}$ $s^{-1}$ (e.g., Chazdon 1988). In regard to the low $I_c$ for the seedlings, our study species may be well adapted to the low light conditions below the closed canopy of tropical rain forests.

***Leaf photosynthetic traits in relation to leaf morphological and biochemical properties***

Height-related differences in photosynthetic characteristics such as $A_{max}$ and $I_c$ are closely related to leaf morphological and biochemical traits. It is well known that $A_{max}$ is strongly affected by various leaf characteristics, such as leaf thickness, leaf mesophyll structure ($A_{mes}/A_a$; Kenzo et al. 2004), and nitrogen content (Evans 1989). In general, age- and size-dependent decline of leaf nitrogen content seems to induce a reduction in $A_{max}$ in tall trees (Koch et al. 2004). However, our results clearly showed that leaf nitrogen content increased with tree height in these tropical canopy species. Although high LMA may also limit $A_{max}$ (Niinemets 2002), dipterocarp canopy trees had a well-developed leaf mesophyll structure, such as a thick palisade layer and high $A_{mes}/A_a$, which is responsible for reduced leaf internal resistance for $CO_2$ diffusion, together with high LMA. These results suggest that high leaf nitrogen and a developed mesophyll structure largely contribute to maintaining a high $A_{max}$ in the upper canopy leaves.

The larger $Chl_{mass}$ and Chl/N ratio in the leaves were related to the lower $I_c$ value, permitting better acclimation under dark conditions in the small-tree stage (Table 2). There was a negative correlation between $Chl_{mass}$ and $I_c$ (Table 3), indicating that higher values of $Chl_{mass}$ contribute to increased light harvesting efficiency at lower light availability (Lambert at al. 1998). In our study, the Chl/N ratio of all species increased with decreasing tree height, and the ratio was negatively correlated with $I_c$ (Table 3); these attributes also contribute to improved light harvesting efficiency in darker conditions.

## Conclusion

Our results suggest that $A_{max}$ is not limited by tree height in tropical canopy tree species. We also found a simple and significant linear relation with tree height for both leaf photosynthetic characteristics (e.g., $A_{max}$) and leaf morphological and biochemical traits, which in turn affect photosynthetic traits (e.g., LMA and $N_{area}$), with some interspecific differences among dipterocarp species. Our study suggests that dipterocarp species can adapt their optimal photosynthetic ability to variable light conditions, from the seedling stage to large adult trees, by changing the morphological and biochemical properties of their leaves.

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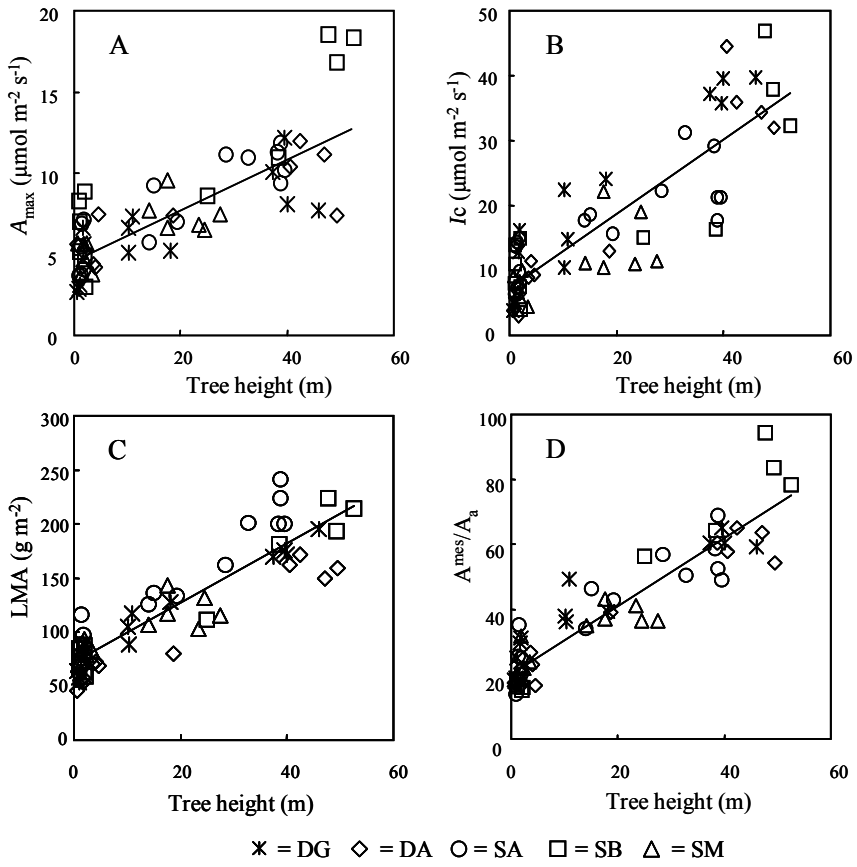


**Figure1**. Relation between tree height and: $A_{max}$ (A), $I_c$ (B), LMA (C) and $A^{mes}/A_a$ (D) (Kenzo et al. 2006). Values are means for each individual across all replicate leaves. The regression lines are: A, y = 4.69 + 0.16x; $r^2 = 0.66$, $P < 0.001$. B, y = 7.34 + 0.57x; $r^2 = 0.74$, $P < 0.001$. C, y = 69.7 + 2.76x; $r^2 = 0.84$, $P < 0.001$. D, y = 20.1 + 1.05x; $r^2 = 0.86$, $P < 0.001$.

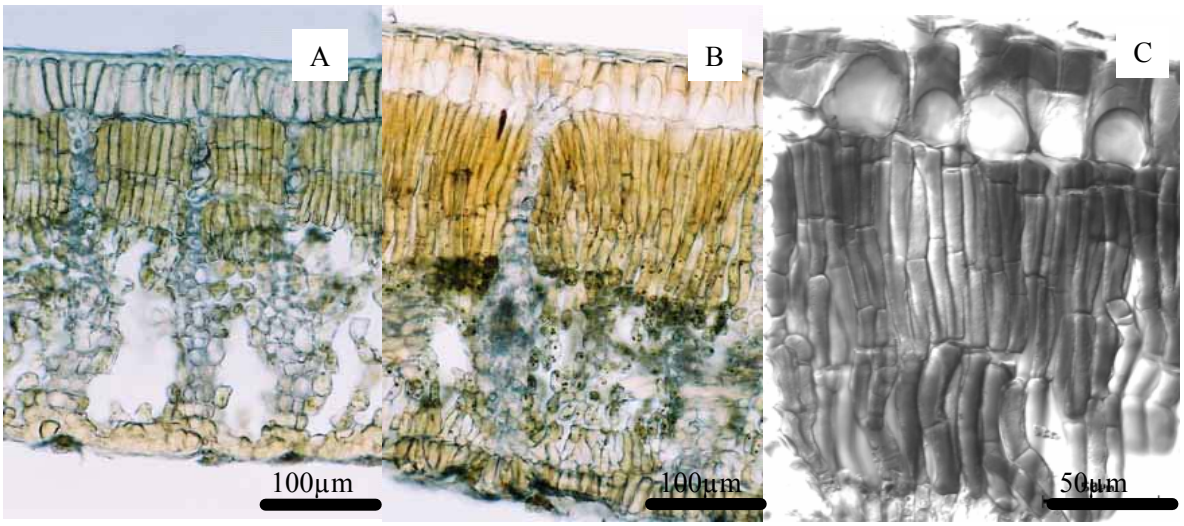


**Figure 2**. Light micrographs of transverse section of *Dryobalanops aromatica* (A) and *Shorea beccariana* (B). Photograph C is the palisade layer of SB at twice the scale (Kenzo et al. 2004).

**Table 1**. Tree species, species code, number of individuals, maximum ($H_{max}$) and minimum ($H_{min}$) tree height. (Kenzo et al. 2006).

| Species | Code | Individual | $H_{max}$ (m) | $H_{min}$ (m) |
|---|---|---|---|---|
| *Dipterocarpus globosus* | DG | 13 | 46.0 | 0.6 |
| *Dryobalanops aromatica* | DA | 12 | 49.4 | 0.6 |
| *Shorea acuta* | SA | 15 | 39.5 | 1.0 |
| *Shorea beccariana* | SB | 11 | 52.5 | 1.0 |
| *Shorea macroptera* | SM | 12 | 27.5 | 0.7 |

**Table 2**. Relation between tree height and leaf characteristics. Values are means for each individual across all replicate leaves (Data from Kenzo et al. 2006).

| Leaf characteristics | Slope | Intercept | $r^2$ | $P$ |
|---|---|---|---|---|
| Leaf thickness (μm) | 4.76 | 206 | 0.69 | 0.001 |
| Palisade layer thickness (μm) | 1.97 | 38.3 | 0.83 | 0.001 |
| $N_{area}$ (mol $m^{-2}$) | 0.0013 | 0.045 | 0.74 | 0.001 |
| $Chl_{mass}$ (mg $g^{-1}$) | -0.05 | 3.48 | 0.49 | 0.001 |
| Chl/N ratio | -0.06 | 5.4 | 0.48 | 0.001 |

**Table 3.** The light-saturated photosynthetic rate ($A_{max}$) and Light compensation point ($I_c$) in relation to leaf characteristics. Values are means for each individual across all replicate leaves (Data from Kenzo et al. 2006).

| Variable 1 | Variable 2 | Slope | Intercept | $r^2$ | $P$ |
|---|---|---|---|---|---|
| $A_{max}$ (μmol $m^{-2}s^{-1}$) | LMA (g $m^{-2}$) | 0.05 | 1.11 | 0.63 | 0.001 |
| | $N_{area}$ (mol $m^{-2}$) | 102.9 | 0.67 | 0.57 | 0.001 |
| | Palisade layer thickness (μm) | 0.08 | 1.67 | 0.76 | 0.001 |
| | $A^{mea}/A_a$ | 0.15 | 1.85 | 0.72 | 0.001 |
| $I_c$ (μmol $m^{-2}s^{-1}$) | $Chl_{mass}$ (mg $g^{-1}$) | -6.98 | 35.5 | 0.51 | 0.001 |
| | Chl/N ratio | -5.06 | 39.3 | 0.43 | 0.001 |